SEPARATION OF XYLOSE FROM GLUCOSE-XYLOSE SOLUTION USING ION EXCHANGE RESINS

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ABSTRACT

In this present study 5 different types of ion-exchange resins were used to separate xylose from glucose-xylose mixture. The resins were strong base cation (SBC), strong acid cation (SAC) and weak acidic cation (WAC) with same ion forms of H^+. Glucose and xylose were measured using single-component isotherms and being measured at 20^{o}C, 30^{o}C and 40^{o}C. The concentration of the glucose-xylose mixtures are 0.5g/L, 1.0g/L and 1.5g/L. The composition of mixture of glucose-xylose also being varies 1:1, 1:9 and 9:1. The entire sample is being tested at different rotation speeds which are 110rpm, 160rpm and 210rpm. The wavelength is set at 620nm for this experiment. All the data obtained from 5 different resins were described in linear isotherms. The result of each resins were compared based on their adsorption capabilities towards those sugar. From the result gathered, Dowex M-31 shows highest separation of glucose from the glucose-xylose mixture. The optimum temperature and rotation is 30^{o}C and 160rpm respectively.

ABSTRAK

Di dalam kajian ini 5 jenis resin pertukaran ion telah digunakan untuk memisahkan xylose daripada campuran glukosa-xylose. Resin adalah kation kuat asas (SBC), kation asid kuat (MPS) dan kation berasid lemah (WAC) dengan bentuk ion sama H +. Glukosa dan xylose diukur dengan menggunakan isoterma satu komponen dan yang diukur pada 20oC, 30oC dan 40oC. Kepekatan campuran glukosa xylose adalah 0.5g / L, 1.0g / L dan 1.5g / L. Komposisi campuran glukosa xylose juga berbeza 1:1, 1:09 dan 9:01. Keseluruhan sampel yang diuji pada kelajuan putaran yang berbeza yang 110rpm, 160rpm dan 210rpm. Panjang gelombang yang ditetapkan pada 620nm bagi eksperimen ini. Semua data yang diperoleh daripada 5 resin yang berbeza telah diterangkan dalam isoterma linear. Hasil setiap damar dibandingkan berdasarkan keupayaan penjerapan mereka ke arah mereka gula. Dari hasil yang dikumpulkan, Dowex M-31 menunjukkan pemisahan tertinggi glukosa daripada campuran glukosa xylose. Suhu optimum dan putaran adalah 30^{o}C dan 160rpm.

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LIST OF ABBREVIATIONS

%	Percentage
^{o}C	Degree Celcius
µm	Micrometer
g	Gram
g/L	Gram per liter
HPLC	High Performance Liquid Chromatography
IER	Ion exchange resin
L	Liter
mL	Milliliter
nm	Nanometer
OD	Optical density
SAC	Strong acidic cation
SBC	Strong basic cation
UV-Vis	Ultraviolet visible
WAC	Weak acidic cation

1 INTRODUCTION

1.1 Motivation and problem statement

Xylose is an intermediate product of xylitol which is a substitute sugar with numerous benefits compare to the normal sugar. It has anticariogenic properties which is a great concern of food industry and biomedical sector. It can be found in woody materials such as saw dust and other hard wood residues (Lei *et al.,* 2007*).*

Xylose can be found around 25% - 35% by weight of the woody biomass and compose inside the hemicelluloses. Woody materials compose of hemicelluloses, cellulose and lignin. Around 50% of cellulose is glucose. Xylitol is hard to harvest abundantly due to the high percentage of glucose in woody materials. So, it is suggested the xylose and glucose to be separated using chromatography method. The glucose is expected to be trapped inside the ion-exchange resin and high percentage of xylose will be recovered in permeate.

Xylose is very useful material in biomedical and bioethanol industries. For bioethanol industries, it can be used as fuel additive which can be produced by fermentation method from agricultural feedstock and crop residues such as corn, sugarcane and other carbon-based sources. Xylose and glucose is the most abundant polysaccharides that can be found in plant cell embedded in cellulose and hemicelluloses. However, xylose cannot be converted efficiently to bioethanol in industrial scale in the present of glucose. (Bi *et al.,* 2010)

The xylose and glucose has almost similar characteristic including its size which is 0.68nm for xylose and 0.72nm for glucose respectively (Sjoman *et al.,* 2007). Ion exchange resin method is the chosen process in separation of glucose-xylose mixtures by using 5 different types of resins. Thus, this research is aims to search the best resin in separating the glucose-xylose mixtures by gaining highest xylose concentration the final sample.

Ion exchange resin separation is very high efficiency method for agricultural, organic analytical chemistry as well as in sugar separation industry since 20th century.(Anand *et al.*,2001). Besides, ion exchange resins (IER) also have been used in industrial processes and biomedical application. (Adam *et al.*, 1935).The common resin media for sugar separation are sulfonated styrene divinylbenzene cation exchange resin which is also the most applied instance in industrial-scale chromatographic separation of glucose and fructose (Al Eid., 2006). The mixture of glucose and xylose is not a usual one since the difference in structure is too little which are 0.68nm and 0.72nm respectively.

In this research, the adsorption process was used by using ion exchange resin (IER) as the separation media to separate glucose and xylose. However, in adsorption process, the ion exchange resin not performing as real ion exchanger but merely acts as an adsorbent (Saari *et al*., 2010). Five different cation resins were used in this research including Dowex M-31, Dowex Marathon MSC, Dowex MAC-3, Amberlite IRN150 and Amberlite IRC86 to determine the separation efficiency of the resins towards glucose-xylose mixture.

1.2 Objectives

1) To separate the xylose from glucose-xylose mixture using ion-exchange resins.

1.3 Scope of this research

The following are the scope of this research:

1) To analyze the effect of temperature at 20^{o}C, 30^{o}C and 40^{o}C. The concentration of the glucose-xylose mixtures at 0.5g/L, 1.0g/L and 1.5g/L. The ratio composition of glucose-xylose mixture at 1:1, 1:9 and 9:1. The effect of rotation speed at 110rpm, 160rpm and 210rpm against the separation of xylose from glucose solution.

1.4 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides a description of physical characteristic of the raw material, xylose and glucose. This chapter also provides a brief discussion about the benefit of xylitol and application of it. A description of the methods that have been use to separate those sugars was included in this chapter. Other than that, a brief description of apparatus and solvent was explained in this chapter.

Chapter 3 gives a review of the method and preparation used in this project. The step was finalized and done accordingly. A detailed step is included in this chapter. The precaution was necessarily taken to avoid accidents from occur.

Chapter 4 is devoted to the results that were obtained from this project. The calculation was included to show the different effect of the parameter.

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.

2 LITERATURE REVIEW

2.1 Xylose

There are several sources of xylose including in woody materials such as saw dust and other hard wood residues (Bi *et al.,* 2009*)*. Because of that reason xylose is commonly obtain from the woody material since it is cheaper and easier to get. Xylose can be found around 25% - 35% by weight of the woody biomass and compose inside the hemicelluloses (Sjoman *et al.*,2007). Xylose is the raw material to produce xylitol by catalytic hydrogenation (Baudel *et al.*, 2005) or microbial conversion (Granstrom *et al., 2008*) which is an alternative high-added-value sweetener with anticariogenic properties of food industry and biomedical sector. Xylitol is a high value sweetener with a five carbon alcohol sugar (Barbosa *et al.*, 1988).

D-xylose chemical formula is $C_5H_{10}O_5$. The chemical and physical characteristic as shown below:

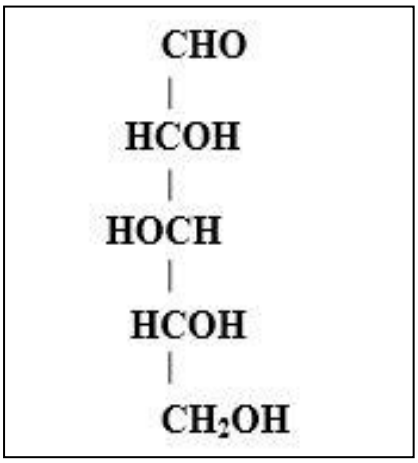

Figure 2.1: D-xylose (Rangaswamy, 2003)

Table 2.1: Physical characteristics and size measures of D-Xylose (Sjoman *et al.*, 2007)

	D-xylose
Molar mass (g mol^{-1})	150.3
pK_a	12.26
Diffusion coefficient at 25^oC (x 10^{-6} cm^2 s^{-1})	7.495
Stokes diameter (nm)	0.65
Equivalent molar diameter (nm)	0.68
Molar volume at normal boiling point (cm^3 mol^{-1})	155.0
Van der Waals volume (cm^3 mol^{-1})	73.6
Hydration number in aqueous solution at 298K	6.8
Solubility parameter	31.0

2.2 Glucose

The glucose can be found in most woody plant. It is the main fuel for the cellular respiration and other biochemical process. Glucose is the main compound that needed inside the cell in order to give energy and it is important for the metabolism of the cell (Wooly *et al.,* 1998). The molar mass of glucose molecule is 180gmol^{-1} and the diameter is 0.72 nm which is slightly bigger than xylose, 0.68 nm. Table 2 shows physical characteristics and some of size measure of glucose.

Glucose also known as aldohexose which contain six carbon atoms in its molecules. The aldohexose sugar can be divided into two isomers known as D-glucose and L-glucose. D-glucose is biologically active but L-glucose cannot be used by cells. Glucose consumed by cell in living things and deposited directly into the bloodstream and transferred throughout the body which helps to supply the energy to the body (Lutz.,2010).

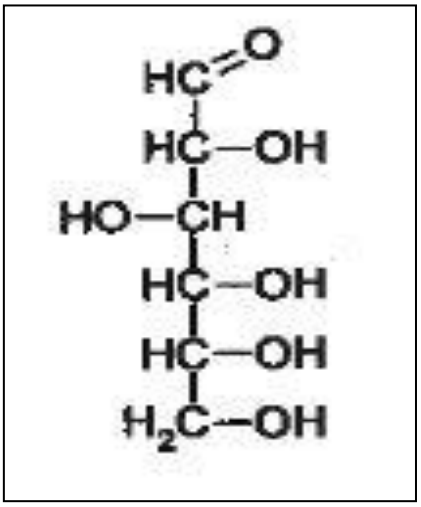

Figure 2.2: D-glucose (Pischetrieder, 2000)

Table 2.2: Physical characteristics and size measures of D-glucose (Sjoman *et al.*, 2007)

	D-glucose
Molar mass (g mol^{-1})	180.6
pK_a	12.43
Diffusion coefficient at 25°C (x 10^{-6} cm^2 s^{-1})	6.728
Stokes diameter (nm)	0.73
Equivalent molar diameter (nm)	0.72
Molar volume at normal boiling point (cm^3 mol^{-1})	189.2
Van der Waals volume (cm^3 mol^{-1})	88.4
Hydration number in aqueous solution at 298K	8.4
Solubility parameter	32.0

2.3 Ion Exchange Resin

Ion exchange chromatography is a technique for separating mixtures based on their charge whether positive or negative. It contains polymeric matrix and functional group with a mobile ion that enable to exchange the ions present in a mixture. The resins normally have spherical shape. According to Srikanth et al., (2010), the resins used contain whether acidic or basic groups. Sulfonic and carboxylic for cation exchangers and quaternary ammonium group for anion exchangers.

The resins also can be classified into 4 types which are strong acid cation, weak acid cation, strong base anion and weak base anion. The schematic diagram of SAC, SBA and WBA as follow:

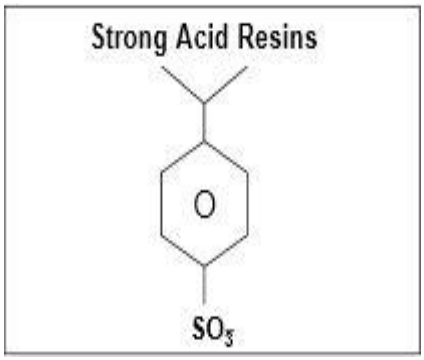

Figure 2.3: Strong acid resin (DOW Company, 2002)

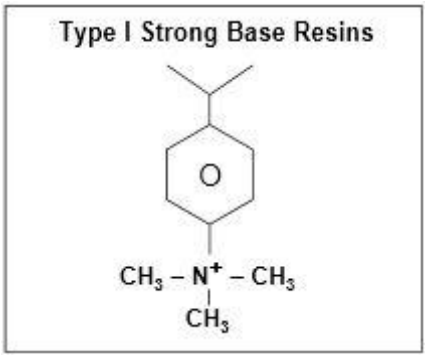

Figure 2.4: Strong base resin (I) (DOW Company, 2002)

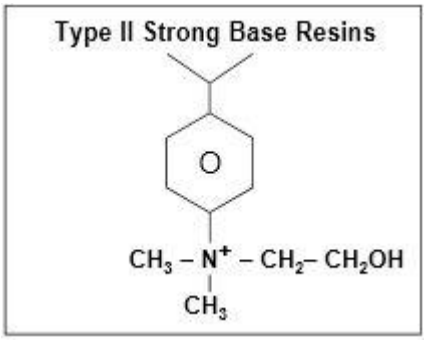

Figure 2.5: Strong base resin (II) (DOW Company, 2002)

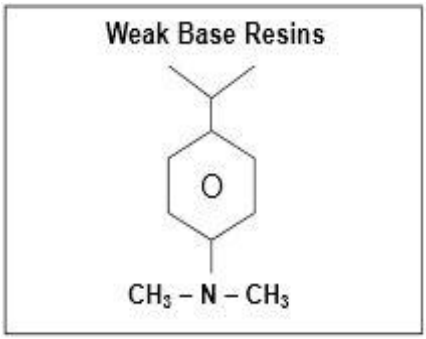

Figure 2.6: Weak base resin (DOW Company, 2002)

The media contain positively or negatively charged functional groups such as K^+, Ca^{2+}, Na^+, H^+ (cation) and Cl^-, NO_3^-, SO_4^{2-} (anion) which attached to the divinylbenzene skeleton. However, 5 resins used in the experiment contain H^+ functional group. The compound that has opposite charge to the functional group will be adsorbed and retained inside the resin. On the other hand, the compound that has similar or no charge will pass through the resin and eluted from the column. The compound adsorbed can be eluted for further investigation.

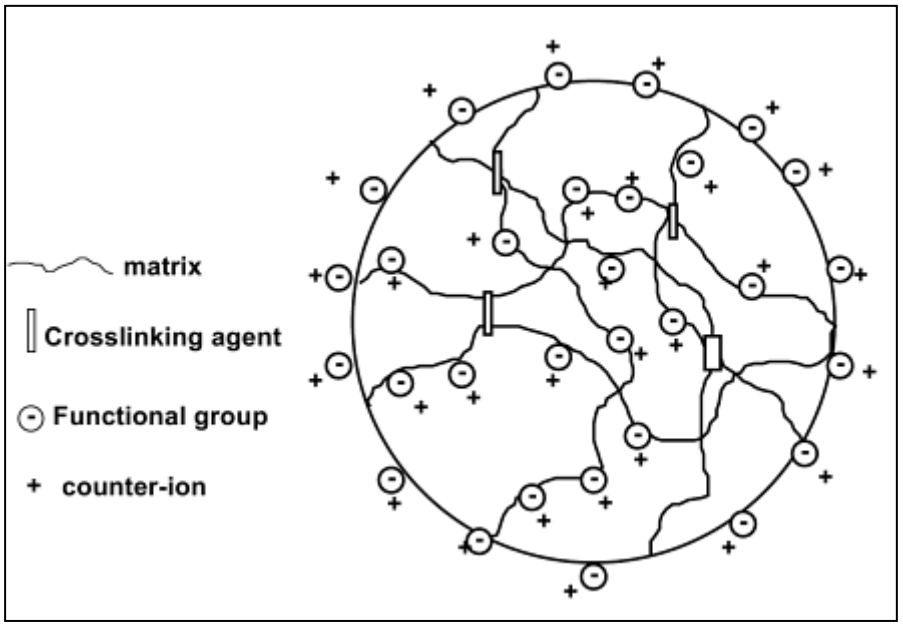

Figure 2.7: Schematic representation of an IER bead (Zaganiaris, 2009)

There 5 different type of resin are being use in the experiment and the list is shown as below:

Table 2.3: Dowex M-31(H+ form)

Limit	140^{o}C max. temp.
Moisture	50 – 54%
Matrix	Styrene-divinylbenzene (macroporous)
Particle size	16 – 40 mesh
	350 – 925 μm
Operating pH	0 – 14
Capacity	1.7 meq/mL by wetted bed volume

Table 2.4: Dowex MAC-3 (H+ form)

Limit	120^{o}C max. temp.
Moisture	44 – 52%
Matrix	Acrylic polymer (macroporous)
Particle size	12 – 50 mesh
	300 – 1200 μm
Operating pH	4 – 14
Capacity	3.8 meq/mL by wetted bed volume

Table 2.5: Dowex Marathon MSC (H+ form)

Limit	150^{o}C max. temp.
Moisture	50 – 56%
Matrix	Styrene-divinylbenzene (macroreticular)
Particle size	24 – 29 mesh
	525 – 625 μm
Operating pH	0 – 14
Capacity	1.6 meq/mL by wetted bed volume

Table 2.6: Amberlite IRN150

Limit	60^{o}C max. temp.
Moisture	49 – 55%
Matrix	Styrene-divinylbenzene (gel)
Particle size	27 – 32 mesh
	580 – 680 μm
Operating pH	0 – 14
Capacity	1.9 meq/mL by dry weight

Table 2.7: Amberlite IRC86

Limit	120^{o}C max. temp.
Moisture	47 – 53%
Matrix	Methacrylic (gel)
Particle size	19 – 26 mesh
	580 – 780 μm
Operating pH	4 – 14
Capacity	4.1 meq/mL by wetted bed volume

2.4 Analysis Method

2.4.1 Anthrone Reaction

This is used for quantitative analysis of sugars. It is a method for determining the amount of carbohydrate in a given sample (Cerning-Beroard., 1975). This method is less expensive compare to High Performance Liquid Chromatography (HPLC). Specific amount of processed sugar mixture was mixed with anthrone solution and heated in boiling water. The sample is case was covered to avoid the liquid from vaporised into the atmosphere. Then the sample was analyzed by using UV-Vis spectrophotometer at wavelength of 620nm.Anthrone was added to the concentrated sulphuric acid to produce anthrone solution. The glycosidic bond in the sugar mixture will be hydrolyzed to form monosaccharides and caused the solution to turn into blue-green colour.

2.4.2 High Performance Liquid Chromatography (HPLC)

HPLC is the best equipment to analyze the carbohydrate in a solution. It has very high sensitivity and accuracy. The solution contained target molecule is injected into the mobile phase and being detected by detector in the equipment. The output of the detector is an electrical signal which displayed on the computer's screen (Lindsay, 1992). The disadvantages of the HPLC are the column is very expansive, short operating life, solvents are expensive and difficult to dispose the used solvent. (McMaster, 2007).

2.4.3 Dinitrosalicyclic acid Method (DNS)

The dinitrosalicyclic acid (DNS) method is the method that gives a rapid and simple estimation of the extent of saccharification by measuring the total amount of reducing sugar in the hydrolysate (Warwik *et al.*, 2007). DNS method also use of xylose standard curve as a standard to determine the amount of reducing sugar released (Bailey *et al.*, 1992). This method is simple, less expansive and suitable to use for large number of samples at a time.

2.5 Separation Process

Separation is a process which compounds or materials of interest are removed from the other compounds in the sample that may react similarly and interfere with a quantitative determination.

2.5.1 Previous works on xylose and glucose separation

1) To separate xylose from monosaccharide mixtures, the adsorption equilibrium of glucose, xylose, and arabinose on five different resins is investigated.The selectivity and adsorption amounts of all the monosaccharide towards 5 different resins were compared. The resins went through the pretreatment process first, and then extraparticle liquid was removed by centrifugation process. The resins and monosaccharides were weighed precisely and poured inside a flask (25mL). The flasks were hermetically sealed and placed inside a tempered shaker at 160rpm at 25^{o}C for 12 hours. Then, the quantification process of the monosaccharide was carried out by using HPLC. The mobile phase used is deionized and degassed water. The dry substance content of the resin was determined by drying until constant weight in a vacuum dying oven at 80^{o}C. (Huajie *et al.*, 2010)

2) The separation was carried out at 60^{o}C for the best performance of the anion-exchange resin. The sucrose-based mixtures were inverted and separated at 45^{o}C to avoid the sugar from caramelized. The flow rates were chosen with previous experience of cation resins. The unit was operated continuously for 10 to 12 cycles for 6 hours to ensure pseudo-equilibrium state was achieved. The product was weighed and analysed at the end of each cycle. The concentration of products was monitored versus time. Then, the column was purged separately to determine the quantity of sugar retained by each column. (Barker *et al.*, 1984)

2) Two types of polymeric adsorbents which are Dowex99 and poly(4-vinyl pyridine)(PVP) was used to recover sugars from corn-stover hydrolyzate. The main component of the hydrolyzate are 5 sugars, glucose, mannose, xylose, galactose, and arabinose, and four impurities, sulphuric acid, acetic acid, hydroxylmethyl furfural (HMF), and furfural. The Dowex99 and the five sugars are packed inside the chromatography column, "center-cut". The sulphuric acid elutes earlier and the other impurities elute later than the sugar. For the column packed with the PVP, the sugars elute earlier than the impurities. The intrinsic adsorption and mass transfer parameter of the sugars and impurities were obtained from elution and frontal chromatography tests on single component. The simulations based on the detailed rate model and single component intrinsic parameter is in close agreement with the experimental elution chromatograms of the hydrolyzate. By using batch chromatography processes the hydrolyzate sugars are recovered and then fermented with genetically engineered yeast. (Xie *et al*., 2005)

3) The feed solutions were made of glucose and xylose with different mass ratios and total monosaccharide concentrations. The ratios of glucose to xylose in the solutions were 9:1, 1:1 and 1:9 respectively. For the monosaccharides concentration it is set at 2, 10 and 30 wt. %. There are 3 types of membranes used in the experiment which are Desal-5 DK, -DL and NF270. The filtration processes were done in total reflux mode (both retentate and permeate were recycled back to the feed tank) at 50^{o}C and the pressure varies from 2 to 40 bar. (Sjöman *et al*., 2007)

4) The xylose and glucose were separated using silica-confined ionic liquid (IL) stationary phase. Five different stationary phases were synthesized and characterized respectively. Compare to NH_2 column, the imidazolium stationary phases exhibit more effective retention to the glucose and xylose. As the concentration of the acetonitrile decrease, the retention factor and resolution of the monosaccharides also decreases. Moreover, the xylose and glucose also being studied on their adsorption behavior. Then, both temperature and mobile phase were optimized in order to improve the performance for the separation of the monosaccharides (Bi *et al*., 2010).

2.6 Data Collection

2.6.1 Ultraviolet – Visible Spectrophotometer (UV-Vis)

After the sample placed in the incubator shaker for 12h, the samples were analyzed with anthrone reaction solution in 1:4 ratio. Then the samples were put in acuvette and the optical density measured using untraviolet-visible spectrometry (UV-Vis). Thus, ultraviolet-visible spectrometry (UV-Vis) method will be used. Ultraviolet-visible spectrometry (UV-Vis) refers to absorption of spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived colour of the chemical involved. In this region of the electromagnetic spectrum, molecules undergo electronic transition. The method is most often used in a quantitative way to determine concentration of an absorbing species in solution, using the Beer – Lambert law. (Williams *et al.*, 2011):

$$A = log10\frac{Io}{I} = \varepsilon . c . L$$

Where A is the measured absorbance with AU as the unit. I_o is the intensity of the accident light at a given wavelength and I is the transmitted intensity. L is the pathlength through the sample and c is the concentration of the absorbing species.
In the experiment the wavelength used is 680nm which is the most suitable as anthrone method is used to determine the sugar concentration in the final sample.

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3 MATERIALS AND METHODS

3.1 Overview

This chapter describes the materials and methods employed for the separation process xylose from glucose solution using ion-exchange resins. It begins with the preparation of sugar samples in different concentration. The sugar mixture undergo the experiment with different rotation speed and temperature Then, follow by incubation and centrifugation process before test it with UV-Vis spectrophotometer.

3.2 Instruments

The anthrone solution was prepared in a 100 mL volumetric flask. 100 mL concentrated sulphuric acid (H_2SO_4) was mixed with 0.2g anthrone powder to produce anthrone solution. Besides, this experiment also used UV-Vis spectrophotometer in determining the isotherms of each samples.

Figure 3.1: UV-Vis spectrophotometer

3.3 Chemicals

Anthrone, sulphuric acid (concentrated), D-glucose and D-xylose were purchased from Sigma Aldrich, Malaysia.

3.4 Experimental Procedure

The main materials used in the experiment are D-xylose and D-glucose. Both sugars were mixed in different concentration (0.5 g/L, 1.0 g/L and 1.5 g/L). The resin was precisely weighed and added with the sugar mixtures. The glucose-xylose mixtures were placed in incubator shaker at certain speed (110rpm, 160rpm and 210rpm). The glucose-xylose mixtures also being tested at different temperatures(20^oC, 30^oC and 40^oC). Moreover, the samples were also tested in different ratio (1:1, 9:1 and 1:9). Then, the samples were allowed in the incubator shaker for 12h.Then, the sample was filtered using 0.2μm filter to separate glucose-xylose mixture from the resin beads. Then, the glucose-xylose mixtures were diluted with ultrapure water in 1:9 ratio (1mL sugar mixture + 9mL ultrapure water). Then, diluted samples were added with anthrone solution in 1:4 ratio (1mL sample + 4mL anthrone). The samples were covered with cap and placed in boiling water for 10 minutes before let it cooled at room temperature. Then, the samples were tested with UV-Vis spectrophotometer at wavelength of 620nm and the curve of each samples with different parameters were constructed.

3.4.1 Standard Preparation

Glucose standard is prepared in 3 different concentrations (0.5 g/L, 1.0 g/L and 1.5 g/L). For each concentration the standard curve is plotted as the guide for the samples result.

The xylose and glucose solution at 1.5g/L is added at different volume and being check using UV-Vis for the absorption wavelength as the standard for the samples.

4 RESULT AND DISCUSSION

4.1 Overview

This chapter discussed the experimental results that carried out in the research work. The material discussed in this chapter includes the effect of different parameters and the sugar concentration after being tested with different conditions. Other than that, this chapter also discussed the experimental result tested using UV-Vis analysis method. Then, each of the results was discussed thoroughly and justified accordingly.

4.2 Glucose Standard Curve

The glucose standard curve with concentration of 0.375g/L, 0.750g/L and 1.125g/L was plotted in figure 4.1 below by using the data in the table 4.1 obtained during the experiment. From figure 4.1, linear equations for glucose concentration were obtained as follows in equation 4.1:

$$Y = mX + C \qquad (4.1)$$

Table 4-1: Absorbance reading from UV-Vis spectrophotometer (Glucose)

Glucose Concentration (g/L)	Optical density (OD)			Average
	1	2	3	
0.000	0.000	0.000	0.000	0.000
0.375	0.407	0.411	0.412	0.410
0.750	0.673	0.670	0.671	0.671

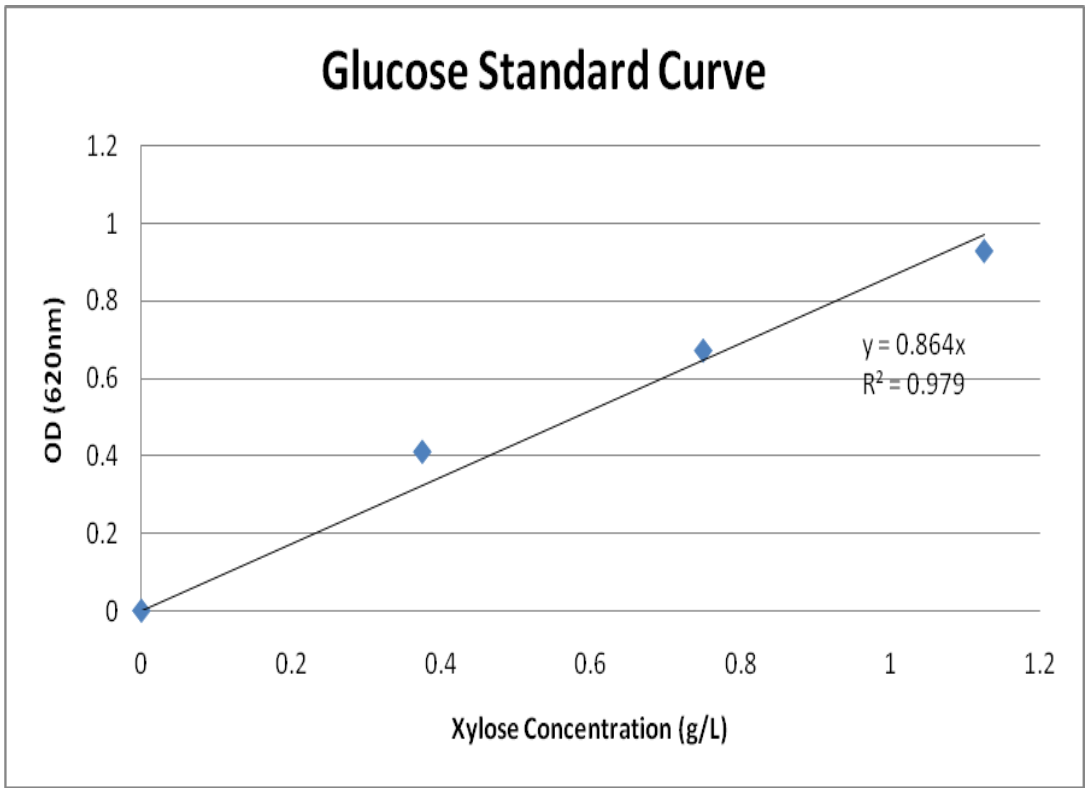

Figure 4.1: Glucose standard curve

4.3 The Efficiency of Resins

Each resins used in the experiment were analyzed for its efficiency. After the separation of glucose-xylose mixture, the supernatant was examined using UV-Vis spectrophotometer. The concentration of xylose in the permeate can be obtained by using the glucose standard curve.

According to Farone and Fatigati (2004), the xylose adsorbed to the resin at higher rate compare to glucose. Thus, it is assumed that the amount of xylose in the permeate must be low for high efficiency resins. The higher the resin efficiency, the lower the amount of xylose in the permeate.

Based on the result obtained from the experiment, its shows that the separation of xylose from glucose-xylose mixture occurred most efficiently at 160rpm and 30^{o}C. The result of the separation shown as below: